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Constant and shifting photoperiods as seasonal cues during larval development of the univoltine damselfly *Lestes sponsa* (Odonata: Lestidae)

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Larvae were reared at 21.5°C from eggs from southernmost Sweden, and fed *ad libitum* to emergence in four different photoperiodic treatments, intended to represent increasing levels of time stress: constant LD 16:8, corresponding to late April (or August) conditions, a shift after about two weeks from LD 16:8 to 19.5:4.5, coarsely simulating late spring, constant LD 19.5:4.5, corresponding to the summer solstice, and a shift from LD 19.5:4.5 to 16:8, coarsely simulating late summer. Mean larval development time significantly decreased in this series: 47.5, 45.2, 43.0 and 39 days (n = 11–13 larvae), respectively. This suggests an ecologically relevant integration of absolute photoperiods and changes in photoperiod, allowing larvae to distinguish if LD 16:8 represented spring or late summer, depending on earlier experience. Thus, rapid development, a long day response during spring conditions, is further speeded up by shorter days during late summer. In early stadia, moulting intervals were uniform, but long days may to some extent have programmed young larvae to develop with fewer moults, thereby increasing development rate. In the last four stadia the principal effect was variation in moulting intervals. Adult size was little affected. Homogeneous conditions and low genetic diversity produced a remarkably synchronous development within treatments, with an emergence span of 5–10 days. Due to low numbers of larvae, derived from a single female, and problems with a switch, the generality of these results would need confirmation.

Keywords: life history; seasonal regulation; photoperiod; time constraint; moulting intervals; growth ratios; dragonfly

Introduction

Photoperiod as a seasonal cue

With its astronomical precision, photoperiod is universally acknowledged to provide the most accurate information for the timing of seasonal events in insect life cycles. In this respect it is superior to the somewhat less predictive temperature conditions, although it is important to point out that photoperiod and temperature are usually interacting (Danks, 1987; Gotthard, Nylin, & Wiklund, 2000; Tauber, Tauber, & Masaki, 1986). There are two aspects of photoperiod which can act as seasonal cues, namely the absolute value of photoperiod, and the rate and direction of changes during the season.

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Most investigators examining photoperiod as a seasonal cue, e.g. for timing of the induction of diapause in seasonal regulation, have traditionally used constant photoperiods in their experiments. In most insects, absolute photoperiods are important cues, but not always changes (Danks, 1987, p. 104; Tauber et al., 1986, p. 118). In Odonata this approach was used in most of the earlier work (e.g. Corbet & Harvey, 1989; Eller, 1963; Ingram, 1971, 1975; Ingram & Jenner, 1976a; Lutz, 1968, 1974; Lutz & Jenner, 1964; Norling, 1971, 1976, 1984a, 1984b; Sawchyn, 1972; Sawchyn & Church, 1973). Only Corbet (1956c), in his initial classical work on the seasonal regulation in *Anax imperator* Leach, used a naturally variable photoperiod for this purpose. Many insects have indeed been shown to respond to changing photoperiods in various complex ways (e.g. Tauber et al., 1986, chapter 5.1.3, p. 118).

In later years the use of photoperiodic regimes mimicking the natural progression has become widespread in studies of larval development in Odonata aiming to test various aspects of life history theory. This applies most notably to time stress, which accelerates larval development in univoltine damselflies, often *Lestes* species (e.g. review in Stoks, Johansson, & De Block, 2008, and references therein; Mikolajewski, De Block, & Stoks, 2015), but also to the study of life history adaptation to different latitudes (e.g. Śniegula & Johansson, 2010; Śniegula, Drobniak, Gołąb, & Johansson, 2014 in *Lestes* and Śniegula, Johansson, & Nilsson-Örtman, 2012; Śniegula, Nilsson-Örtman, & Johansson, 2012 in *Coenagrion* species).

The photoperiodic regime approach is often ideal, in particular in studies of time constraints, where different seasons are easily simulated by phase shifting the photoperiodic regimes. The proximate responses can then be treated as the workings of a black box, where we do not need to know how it operates, for example if the photoperiodic responses are to absolute values, to changes *per se*, or to a combination thereof. Although changing photoperiods are natural, this approach may also be problematic. It is notoriously difficult to tease apart the two kinds of proximate responses when photoperiod is changing (Corbet, 1999, p. 229; Danks, 1987, p. 110). When used in latitude studies, time/phase differences are often inadvertently introduced between different test groups.

Virtually nothing is known about the relative influence of changes and absolute photoperiod for the responses in *Lestes sponsa* (Hansemann) or its congeners, which are important model species in the above studies. Even among other dragonflies relatively little is known about these details (cf. Corbet, 1999, p. 229; Norling, 1976, p. 251, figure 3; 1984b, p. 542, figure 7; 1984c, pp. 134–135). In odonates there is probably an integration of responses to temperature, absolute photoperiod, and changes in photoperiod, in a pattern specific for different larval sizes at different times, thereby adapting development to different seasons. It is often producing a two-step photoperiodic response in species with overwintering larvae (e.g. Corbet, 1999, p. 230; Norling, 1984c), a pattern known from both suborders of Odonata. Here a short-day long-day shift is crucial to initiate development to emergence. Odonata probably have a common physiological toolbox for translating environmental cues to appropriate responses (black box contents), and therefore a related complex of interactions can occur in the egg-overwintering univoltine *Lestes*.

As suggested above, the proximate responses to photoperiod and temperature may be regarded as the working of a black box, processing information about seasonal progress. The responses involved may not be perfect, but operate well enough to have been favoured by selection under naturally encountered conditions. When experimental conditions are outside the normal ecological range of conditions, the responses may not have been subjected to previous selection, and the black box may not produce an ecologically meaningful output.

One possible example of such situations in Odonata is prolonged periods of constant photoperiods, in particular if at a uniform temperature, and present during the whole time from the egg stage to emergence. This is a common approach in many studies for other purposes (e.g. Nilsson-Örtman, Stoks, De Block, & Johansson, 2012; Stoks & De Block, 2011). It does in fact partly apply also to the present study, and may give static seasonal information lacking cues

of seasonal progress needed for normal development, particularly in later stadia where regulatory responses may be strong. Photoperiodic regimes where absolute photoperiods and changes are strongly deviant from what is found at the source latitude of the population, and unnatural long-term temperature–photoperiod combinations, may also belong here. Such situations are of course not predetermined to produce irrelevant responses, but they just may do so, depending on the evolutionary background and on what physiological mechanisms are operating.

One particularly intriguing example is a time constrained (late hatching) and food deprived group of *Lestes sponsa* (Johansson, Stoks, Rowe, & De Block, 2001). Instead of trying to accelerate development and emerge as small adults as expected, these developed slowly, and emerged as very large adults as late as early November photoperiodic time, but still at summer temperatures. As discussed by the authors, it may point towards the evolution of a cohort split and partial semivoltinism in this obligately univoltine species. A possible proximate explanation, not discussed by the authors, is that the autumn photoperiods, which may have been encountered by relatively small larvae, and at summer temperatures, produced an early spring response. This does not invalidate the author's discussion, presented at a higher level of abstraction.

The life history and seasonal regulation of Lestes sponsa

Lestes sponsa is the most common lestid in Sweden, and it is even found above the Arctic Circle (Billqvist, Smallshire, & Swash, 2012; Śniegula et al., 2014). It has an obligately univoltine life cycle like other European *Lestes* species, and survives winter as a resistant diapause egg, containing a fully developed embryo, virtually ready to hatch. After a synchronous hatching in spring, larval development takes only a couple of months (Figure 1b–d; Corbet, 1956a), and emergence is often at its peak during early July in southern Sweden (own observations), although it has occasionally been recorded on the wing as early as the first week in June (Artportalen, 2015).

The obligatory egg diapause, which is pivotal in the seasonal regulation, has been studied in this species in Britain (Corbet, 1956b). In Canada a particularly detailed study of *L. disjunctus* Selys and *L. unguiculatus* Hagen, both closely related to *L. sponsa*, has been made (Sawchyn, 1972; Sawchyn & Church, 1973; Sawchyn & Gillott, 1974). These two species differed little from each other, and are, as far as is known, a good model for *L. sponsa*. All these studies are expertly summarized by Jödicke (1997). The following is, in most details, based on the Canadian studies.

After oviposition during summer the advanced overwintering diapause stage is reached in a couple of weeks, and visible development is halted in the fully formed embryo. Termination of diapause was described to occur in two steps, controlled by temperature and photoperiod, respectively. The first step, the thermal phase, is most rapidly finished after some weeks at autumn temperatures, preventing premature hatching at high temperatures. Thereafter, in the photoperiodic phase, higher temperatures and long spring photoperiods rapidly (4–8 days) terminate diapause, whereas short days still maintain diapause, ensuring that hatching is postponed until spring (mean air temperature *c*. 10°C; see also Corbet, 1956b). Another requirement for starting post diapause development was found to be wetting, which means that eggs in plants above water delay hatching until submerged.

In *L. sponsa* in Japan, an adult pre-reproductive diapause has been shown to ease the load on the egg diapause in the warmer central and southern parts of the country, prolonging the period between emergence and reproduction (Uéda, 1978, 1989).

In all these species both larval development and the following reproduction must be finished within the season. Hence, in colder climates, late developing larvae are under particularly strong time stress to be able to emerge in time to reproduce successfully and allow eggs to enter diapause

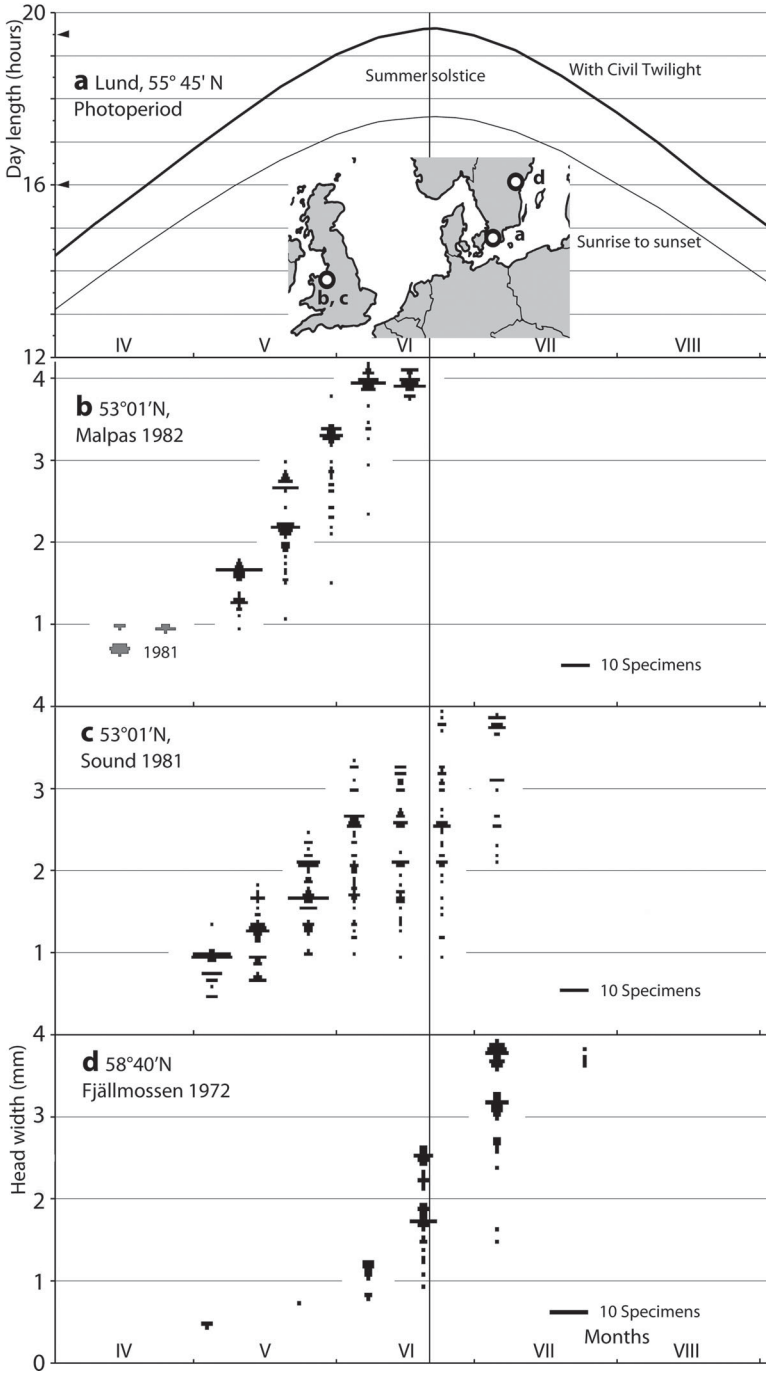

Figure 1. Photoperiodic changes during the recorded life history of populations from Britain and Sweden of *Lestes sponsa* shown on a common timescale. (a) Spring and summer photoperiods at 55.75° N, the source latitude in the present study. Experimental photoperiods are indicated by arrows. The map shows the location of the different populations, where (a) is the populations in the present study, and (b) to (d) refer to the graphs below. (b, c). Larval growth in two adjacent (*c*. 15 km) localities in Cheshire, England, 53.02° N, redrawn from data in Pickup et al. (1984): (b) small weedy pond at Malpas; (c) shallow vegetation-choked *Typha*-dominated pond at Sound. (d) Summary of larval growth in two somewhat confluent bog pools at Fjällmossen, southern Sweden, 58.67° N. Previously unpublished by-product from a study of *Coenagrion hastulatum* (Charpentier); see Norling (1984a). The slow growth in May was due to unusually cold weather following a short early period of summer temperatures. Some larvae in early July were close to emergence, and in late July the few remaining ones all showed signs of imminent emergence.

before winter. Mediated by photoperiodic cues, development is accelerated in such larvae, and they often emerge as smaller adults (Johansson & Rowe, 1999; review in Stoks et al., 2008). This effect on development rate appears as a moderately variable continuum of rates, seemingly different to the often all or none, switch-like induction of diapause.

The life history of this species is relatively well known from field studies. In Figure 1, life history graphs from three places from two latitudes are compared with the photoperiodic regime of the area of the present study. The local photoperiodic regimes for these populations are of course different, but in phase. The fastest-growing British population (Figure 1b) grew almost exclusively in increasing photoperiods. On the other hand, the slow-growing, very asynchronous British population, and the Swedish population (Figure 1c, d) passed the solstice mainly in mid-stadia, and late stadia definitely encountered decreasing photoperiods.

That late stadium *L. sponsa* larvae may encounter still shorter late-season photoperiods is shown by the occasional occurrence of large larvae in autumn, too late to emerge (Valtonen, 1982, in Finland), and such larvae have even been shown to overwinter in Britain (Warren, 1988). The slow British population (Figure 1c), in a *Typha*-pond, was so asynchronous that the authors even considered an asynchronous hatching (Pickup, Thompson, & Lawton, 1984). This could actually have happened if some parts of the *Typha* plants, where females often oviposit high above water level, as was observed during the present study, were not wetted until after the usual hatching period. This is a possible but yet unproven background for many late appearing small *Lestes* larvae, which must be under considerable time constraint. Also local low temperatures, low food, and inappropriate "outside the box" responses to very short days could contribute to late larvae. Another possible explanation is non-diapause egg development or premature diapause termination, known from other species at premature wetting in the Mediterranean (Rota & Carchini, 1988).

The exact nature of the photoperiodic cues and responses, and at what time during development *Lestes* larvae are sensing the cues, and how and when the responses are appearing is essentially unknown, since most studies only have recorded the time between hatching and emergence. The study of De Block, McPeek, and Stoks (2008) is a partial exception discussed later. The present study attempts to address these questions in a small and simple experiment on material from Lund in southernmost Sweden, 55.75° N, 13.02° E.

Hypotheses

In studies on time constraint in other insects, constant long summer days accelerated development more than shorter spring days for the attainment of a midseason adult emergence. Conversely, shorter days were the most time constraining for autumn events (Nylin & Gotthard, 1998). Parts of some older constant photoperiod results on Odonata were actually in both these categories, although time constraints were not discussed at the time (e.g. Norling, 1971, figures 8–11, summer accelerating response in *Aeshna viridis* Eversmann; Norling, 1984a, p. 437–442, both summer and autumn responses in *Coenagrion hastulatum* (Charpentier)). Considering the sometimes late larval development in *L. sponsa*, both situations could be relevant for the timing of emergence in this species. Thus, there may not be a single simple response to absolute photoperiods in all larval stadia in *L. sponsa*.

A possible hypothesis is that the absolute value of photoperiod during early stadia, when long days equal time stress, determines the rate of development for the whole larval life, but this appears inflexible, as it does not include conditions during late larval life. In late stadia, long days are a relatively normal situation, and should indicate a development "on schedule", whereas spring photoperiods occur in both spring (time surplus, "ahead of schedule") and late summer

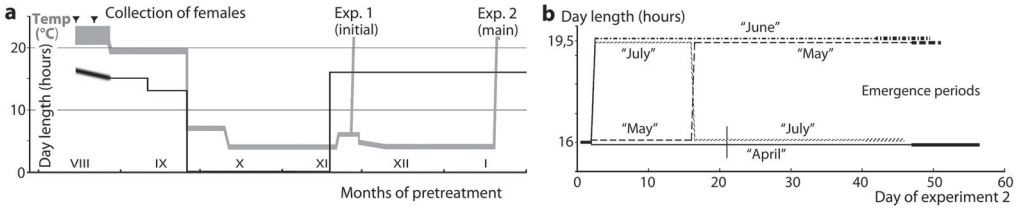

Figure 2. Graphical presentation of treatments of eggs and larvae. (a) Egg treatment before experiments, where temperature is shown in grey, and photoperiod (photophase) in black. Note that temperatures during pretreatment were not strictly controlled, and could usually vary 1–2°C. (b) Photoperiod treatment of the four groups (16-16 or "April" to 19-16 or "July") in experiment 2, the main experiment. The graph starts on the day eggs were brought out from winter temperatures (day 0). Hatching peaked just after the distribution into experimental photoperiods on day 2. The thickened parts of the lines show the emergence periods. The vertical line shows the detection and correction of the faulty switch at LD 16:8 on day 21. Legend: solid lines 16:16 "April", simple broken 16-19 "May", mixed broken 19-19 "June", obliquely hatched 19-16.

(extreme time constraint, "behind schedule"). To be ecologically relevant, responses to these photoperiods in late stadia should then depend on if they occur after a period of longer days (late summer) or shorter days (spring), which means an effect of changes.

This, and how and when responses appear, was tested with the simplest possible approach including two constant photoperiods, LD 16:8 and LD 19.5:4.5, both at a constant 21.5°C. In the source area, with the two periods of Civil Twilight added as a reasonable approximation of photoperiodic perception (Beck, 1968), these photoperiods are appropriate for late April/late August, and the summer solstice, respectively (Figure 1a). To at least crudely mimic different timing within the photoperiodic regime, there were four treatment groups (Figure 2b), a spring "April" group at constant LD 16:8 (called 16-16), a late spring "May" group shifting after two weeks to LD 19.5:4.5 (called 16-19), a midsummer "June" group at constant LD 19.5:4.5 (called 19-19), and finally a late summer "July" group shifting after two weeks to LD 16:8 (called 19-16). This is an ultimate extension of the stepwise adjustments every seven or 10 days mimicking photoperiodic regimes in many studies (e.g. De Block & Stoks, 2003; Śniegula & Johansson, 2010).

If absolute values of photoperiod are the sole determinant, the 16-16 group should be slowest, and the 19-19 group the fastest, with the 19-16 and 16-19 groups in between, their ranking determined by the relative role of the responses in stadia before and after transfer. If larvae can integrate both absolute photoperiod and changes in photoperiod in an appropriate manner, and if the very large shifts can simulate a gradual transition, development rate should increase successively from the 16-16 ("April") group to the 19-16 ("July") group, reflecting an increasing time constraint with the advancing season.

According to the egg studies discussed under seasonal regulation, it was hypothesized that the desired synchronous hatching of eggs at the start of experiments, as happens in the field during spring, should be attained by a simulated winter pre-treatment at various low temperatures for two months (the thermal phase) and, at the end, combining this with a spring photoperiod (the photoperiodic phase; Figure 2a), and finally a temperature increase.

Materials and methods

Collections, egg pretreatment and equipment

Females of *Lestes sponsa* were collected on 15 and 22 August 2013 from two populations just NW of Lund, Sweden (*c*. 55.75° N, 13.13° E), separated by 2 km of mainly agricultural land.

One population (A) occurred at a relatively large (c. 140 × 30 m) productive pond with a high diversity of plants, dragonflies, and other invertebrates. It is located in the northern part of the local nature reserve "Nöbbelövs mosse".

The other population (B) occurred at a small (*c*. 20 × 15 m) very uniform roadside pond choked with *Typha latifolia* and with virtually no open water. The *Lestes* population was dense, but there were few other dragonflies. After the sampling in 2013, most of the *Typha* was removed, and open water restored, soon colonized by *Chara* mats.

Adult samples from these populations were taken, preserved in alcohol, and sent to Szymon Śniegula in Poland for use in another study, where head widths were measured by digital callipers. Because of an exceptionally small body size of the 2013 population B adults, additional adults were taken in early August 2015, in connection with another study, and preserved similarly. The latter specimens were measured with a calibrated ocular micrometer in a Wild M5A dissection microscope, and were placed in a gimbal-supported vial on a movable mechanical stage for precise adjustment to the micrometer scale. The scale was used in a vertical position in the field of view to avoid any lateral nonlinearity often present in this type of instrument. This optical method is probably closely equivalent to the similarly calibrated photographic measurements on experimental animals described below.

The captured females from 2013 were allowed to oviposit in wet paper in uncontrolled indoor conditions. The eggs were then kept under these conditions in order to develop and enter diapause until September, when temperatures and photoperiod were decreased (Figure 2a). The eggs were finally stored at *c*. 4°C and darkness. Later during cold storage LED lamps provided a spring photoperiod of LD 16:8.

Experimental equipment consisted of two enclosed aluminium water baths, both with a temperature of 21.5°C, usually kept within ± 0.2°C. The average difference between them is estimated not to have exceeded 0.1°C. Light was provided with LED lamps, controlled by switches giving LD 16:8 and LD 19.5:4.5 in the two containers. Unfortunately the limited space available necessitated the use of few larvae, housed under cramped conditions in 30, and later in 60 ml transparent plastic cups, placed in the water on a grid above the bottom. Water level was kept at 15–20 mm. The arrangement should have allowed visual contact between larvae in adjacent cups.

Experimental treatments

Two experiments were performed after each other. The first was small and aimed to test methods, and to obtain a general orientation of responses under the used experimental conditions. This experiment (experiment 1), with eggs from a single female from population A collected on 22 August, started in the morning of 27 November 2013 (time zero, on day zero), allowing eggs to acclimate until the evening. The eggs were then divided between the two photoperiods and later produced 18 hatchlings. The experiment was mainly run with one group in each photoperiod, but three larvae were transferred from LD 19.5:4.5 to LD 16:8 on day 19.

The main experiment (experiment 2), with eggs from a single female from population B collected on 15 August, was started in the morning on 20 January 2014 (time zero), and eggs were allowed to acclimate to 21.5°C until next morning at LD 16:8. Eggs intended for LD 19.5:4.5 were, for logistical reasons, not transferred to this photoperiod until day 2, during the inspection interval when the first two hatchlings appeared. 71 eggs hatched, but only 60 larvae could be accommodated in the water baths, and so some late hatchlings had to be excluded. The larvae were subsequently divided into the four groups mentioned above (see Figure 2b). The transfer between photoperiods of the 16-19 and 19-16 groups took place on day 16, since the transfer on day 19 in the initial experiment did not seem to influence development.

The number of larvae in each group at the time of transfer was 12 (14 in 19-19), due to some initial mortality. The time of transfer was thus 14 days after the eggs were distributed to the experimental photoperiod, which just preceded hatching. At transfer time the larvae were moulting into stadium 6 (the prolarva counted as stadium 1) and a head with of 1.5–2.0 mm (Supplementary Figure S2), a size common at the solstice in the Swedish population from 58.67° N (Figure 1d).

On day 21 during the second experiment an externally invisible error in the switch for LD 16:8 was discovered, which produced 15 min of darkness 45 min after light-on (see Figure 2b). This error could have appeared during the experiment, or been present from the outset, evading detection in the functional check. The switch was exchanged and relegated to more mundane tasks, where it later broke down, suggesting a successive degradation.

The main food was richly supplied *Artemia* metanaupliae. Due to the short life span of *Artemia* in fresh water (a few hours), and the short moulting intervals in *L. sponsa*, larvae were fed and inspected for moults twice daily, at about 0700 to 0900 in the morning and 1800 to 2000 in the evening. At about transfer size (*c*. 1.7 mm head width) when larvae could handle bigger food items, *Artemia* was supplemented with, and in the final stadium replaced by, commercially available enchytraeid or tubificid worms. This ensured an *ad libitum* feeding regime essentially adapted to larval size. The vials were, when needed, cleaned from dead food items, faeces and *Tubifex* slime, and water was changed when turbid.

Measurements and observations during experiments

After moulting, head width was measured on the following day. For measurement, larvae were carefully transferred to a flat vial, where they were moving freely. The larvae were photographed with a telecentric (parallel perspective) arrangement of two old 58 mm Helios-44-2 Soviet made camera lenses for a Zenith 35 mm SLR camera, mounted on a Panasonic G5 (Panasonic Corporation, Osaka) digital camera, giving a precise and distortion free 1:1 image on the 17.3 × 13 mm 16-megapixel camera sensor. A mixed transmitted and incident illumination was used to provide both contrast and body details. Care was taken only to use pictures of larvae sitting with their transverse head axis perpendicular to the optical axis, and to prevent any object breaking and deforming the surface film near the larva. Measurement was performed in Photoshop Elements 9™ by (Adobe Systems Inc., San Jose, California) pasting the image of a micrometer scale taken with the same optics into the pictures as a separate layer, which was easily moved around to take measurements (Figure 3). From measurements in some redundant pictures, errors were estimated to be less than 0.03 mm in large larvae, and 0.02 mm or even better in smaller ones. Emerged adults were also photographed alive, but held in position directly on the stage.

In final stadium larvae, the timing of two distinct points in development, probably closely equivalent in all Odonata, were recorded. These were the folding of the costal rib relatively soon after apolysis about half way to emergence, and the pigmentation of the two rows of short spines along the costal rib (Figure 4a, b). The latter point indicated emergence within two days, and was well correlated with the histolysis of the labium. These essentially qualitative landmarks of development were easily observed without handling the specimens, and they appeared unambiguously and rather suddenly within a few hours. Emergence of two larvae dying prematurely (one in each experiment) was estimated from these data with an estimated error less than ± 1 day. For calculation of total development time in experiment 2, where some larvae died early in F-0, the duration of the whole F-0 was estimated for three larvae in the 19-16 group from typical durations in the group (± 1 day), and for one in the 16-16 group (± 1.5 days).

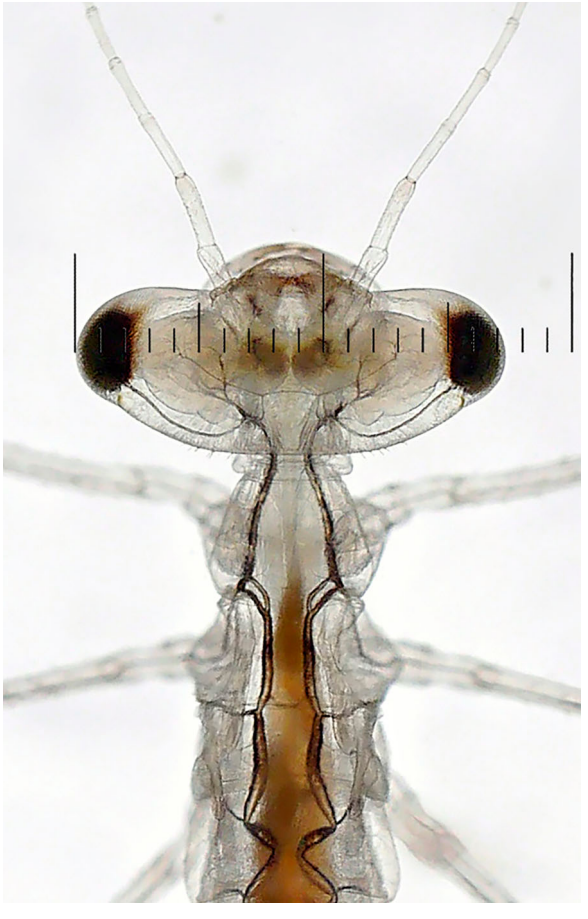

Figure 3. Photo of sixth stadium larva (head width 1.73 mm) showing measuring method.

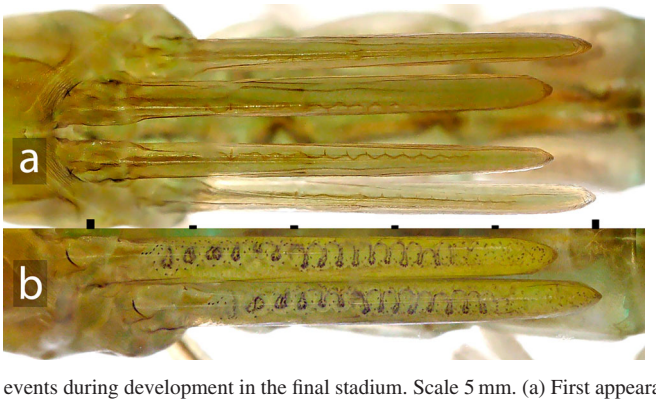

Figure 4. Recorded events during development in the final stadium. Scale 5 mm. (a) First appearance of the folding of the adult costal rib within the larval wing sheath. (b) Relatively early stage of the pigmentation of the spines on the costal rib (dark dots). Pigmentation is yet somewhat weak and is not yet complete at the wing tips. The first spots to appear are those at the very base of the wing.

Analyses

Since all treatments included males and females in about equal numbers it was tested with ANOVA whether sex had an effect on duration of development. Because differences were not significant ($F_{1,47} = 2.575$, $p = 0.1154$) sex was not considered in the following analyses. Due to the small numbers of larvae in experiment 1, this experiment was not statistically analysed.

Effects of treatments in experiment 2 were tested by applying one-way ANOVAs followed by pairwise analyses (Tukey's Q). Response variables were duration of development from experimental start until emergence and from hatching to stadium 6 and stadium 8. Further on, the duration of each larval stadium and the growth ratio (size increase per ecdysis) was tested. This was done only for larvae with 10 stadia to avoid effects of different stadia numbers. Here repeated measures ANOVAs were used with durations of each stadium as repeated response variable. This was followed by univariate ANOVAs for treatment and each single stadium to identify when during development effects appeared.

Differences in numbers of specimens with low and high numbers of stadia were tested with contingency table analysis. For this, data from experiments 1 and 2 were pooled and used together, although derived from different females and with some differences in pretreatment.

Results

Hatching

Hatching was extremely fast and synchronous, and the hatching times of those specimens surviving to late stadia are included in Figure 5. In experiment 1 this comprised 16 of 18 hatchlings, and in experiment 2, 50 out of 71. In both experiments all except two stragglers hatched during day 2–4 after start, indicating that diapause termination was complete (note that day 4 ended in the morning of day 5; cf. legend to Figure 5). The last single straggler hatched during day 6. No differences in hatching time between photoperiods were evident. However, hatchling size was bigger in experiment 1, in particular in the longer day treatment (Supplementary Figure S2).

Mortality

Of the initial 60 hatchlings entered in experiment 2, 50 larvae reached transfer time, and 41 emerged successfully, and three additional ones reached emergence time but only partly emerged. Two of these drowned, and one was stuck in the exuvia during emergence. Part of the mortality was due to handling accidents during cleaning and measuring. Of the 18 hatchlings in experiment 1, 14 emerged successfully.

Emergence time and larval development time

An effect of photoperiod, reflecting the simulated time constraint, was seen in emergence time (Figure 5, also indicated in Figure 2b) and larval development time (Table 1). Due to the short hatching period, the emergence dates showed essentially the same relationship to each other as larval development time. In experiment 1, the six larvae at constant LD 19.5:4.5, and the three with transfer from LD 19.5:4.5 to LD 16:8 on day19, were not different (Table 1), and are therefore treated as one group below.

In experiment 2 the timing of emergence from experimental start differed significantly between the groups (ANOVA, $F_{3,47} = 37.83$, $p < 0.001$). The 19-16 group, with a decrease in photoperiod, was significantly earlier (mean ± SD: 42.5 ± 1.5 days, $n = 12$) than the other

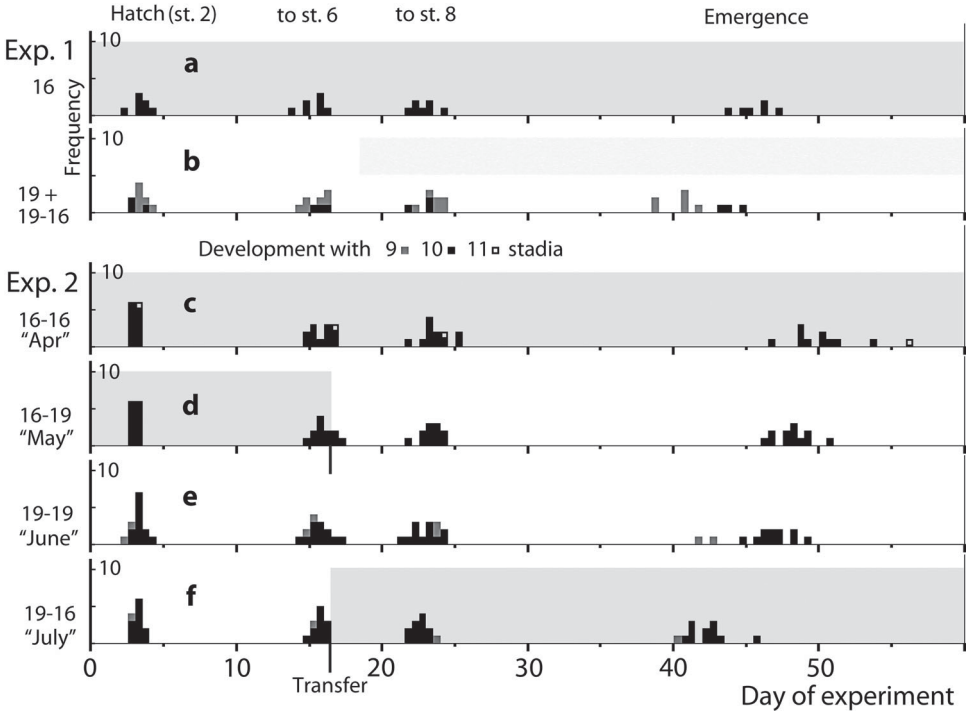

Figure 5. The timing of hatching, entry into stadium 6 (including prolarva), into stadium 8, and emergence, in the different treatments of experiment 1 (a, b) and experiment 2 (c, d, e, f). Eggs were brought out from cold storage at time 0, and hatchings and moults are shown for the inspection interval of half a day preceding the record of the event. Development in LD 16:8 is indicated with a light shade. In four specimens dying in F-0, the timing of emergence has been estimated from the typical F-0 duration in the respective group. The error is unlikely to exceed ± 1 day. Note that each individual larva is represented four times in the graph.

Table 1. Total larval development time (days from hatching to emergence) in the different experimental groups.

		Experiment 1			Experiment 2		
First photoperiod	Second photoperiod	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*
16	16	42.25 ± 1.74	39.5–44.0	6	47.45 ± 2.47	44.0–53.0	11
	19.5	—	—	—	45.17 ± 1.29	43.0–47.5	12
19.5	19.5	38.17 ± 2.14	35.5–41.0	6	43.04 ± 1.83	39.0–46.0	13
	16	38.00 ± 2.78	35.5–41.0	3	39.04 ± 1.39	37.5–42.5	12

Experiment 1, 19-16: Final stadium duration estimated for one of three specimens from the average in the 19-19 and 19-16 groups
Experiment 1, 19-16: Final stadium duration estimated for one of three specimens from the average in the 19-19 and 19-16 groups.
Experiment 2, 16-16: Final stadium duration estimated for one of 11 specimens from the average in the group.
Experiment 2, 19-16: Final stadium duration estimated for three of 12 specimens.

three treatment groups (Tukey's Q, $p < 0.001$). The 16-16 group (50.7 ± 2.6 days, $n = 11$) was significantly later than the 16-19 and 19-19 groups (48.4 ± 1.3 and 46.5 ± 2.1 days, $n = 12$ and 13, respectively) (Tukey's Q, $p < 0.05$), whereas between the latter two groups (16-19 and 19-19) there was no significant difference (Tukey's Q, $p = 0.09$). Compare larval duration in Table 1.

In the smaller and preliminary experiment 1, development was on average faster than in experiment 2; in particular the difference was notable in the constant LD 16:8 photoperiod (Figure 5a, c, Table 1). The nine specimens initiated at 19.5:4.5 in experiment 1 had a mean larval duration

Table 2. Number of larvae developing with 9, 10 and 11 stadia in the experimental groups.

		Stadia in experiment 1			Stadia in experiment 2		
First photoperiod	Second photoperiod	**9**	**10**	**11**	**9**	**10**	**11**
16	16	0	7	0	0	11	1
	19.5	-	-	-	0	12	0
Sum		**0**	**7**	**0**	**0**	**23**	**1**
19.5	19.5	4	2	0	2	12	0
	16	2	1	0	1	11	0
Sum		**6**	**3**	**0**	**3**	**23**	**0**

of 38.1 ± 2.2 days (emergence at 41.7 ± 2.1 days), and the two fastest specimens required only 35.5 days from hatching to emergence. At the other end, the slowest specimen in the 16-16 group (constant 16:8) in experiment 2, required 53.0 days.

Development within treatments was remarkably synchronous, and the emergence times and development times of the 16-16 and 19-16 groups in experiment 2 did not overlap (see also Figure 2b and Supplementary Figure S1). The emergence period of the constant photoperiod groups was longer (within 10 and eight days) than the groups with transfers (within five and six days; Figures 2b, 5), but this relied to a high degree on a few individuals.

When are differences appearing? Number of stadia and moulting intervals

The number of stadia (including the prolarvae) varied from nine to 11 (Table 2). Not unexpectedly, nine-stadia larvae were among the first to emerge (Figure 5b, e, f), and the 11-stadia one the last (Figure 5c). A majority of the larvae developed with 10 stadia. Development with 9 stadia occurred only in groups starting with the June photoperiod, in total nine specimens (Table 2), and the sole specimen developing with 11 stadia started with the April photoperiod. The numbers of specimens with nine versus ≥ 10 stadia differed significantly between the four treatments (contingency table, DF = 3, $\chi^2 = 8.813$, $p = 0.032$). Also, six of the nine larvae with nine stadia were among the generally faster growing larvae in experiment 1, and the difference in emergence time/development time between groups in experiment 1 was highly dependent on this.

Hatching time differed little between the larvae assigned to the different treatment groups (Figure 5). Also the entry into stadium 6 was closely simultaneous among groups, and took place close to the time of the transfer between photoperiods in experiment 2. However, there was a slight but significant difference in the time from hatching (ANOVA, $F_{3,49} = 3.651$, $p = 0.019$) between the treatments, which mainly derived from a significant difference between the 16-19 and 19-19 treatments (Tukey's Q, $p = 0.034$), whereas there were no differences between all other treatments ($p > 0.12$; Figure 5). With the time from hatching to stadium 8 the variance between the treatments became more pronounced (ANOVA, $F_{3,49} = 6.189$, $p = 0.001$) with a significantly longer time in the treatments starting at the 16-hour day length (Tukey's Q, $p < 0.05$), with the exception of the comparison between the 16-19 and the 19-19 treatment ($p = 0.16$).

However, the size of some of the sixth stadium larvae (near transfer) differed between groups (Supplementary Figure S2). The larvae with nine stadia, all from the groups which were started at LD 19.5:4.5, were not unexpectedly each bigger than all the rest, and the 11-stadia one was among the smallest. Photoperiod had thus little influence on the timing of the early moults (see also the graphs in Supplementary Figure S1), but, an early, pre transfer-time influence on growth ratios is at least consistent with the scarce data, long days reducing the total number of moults.

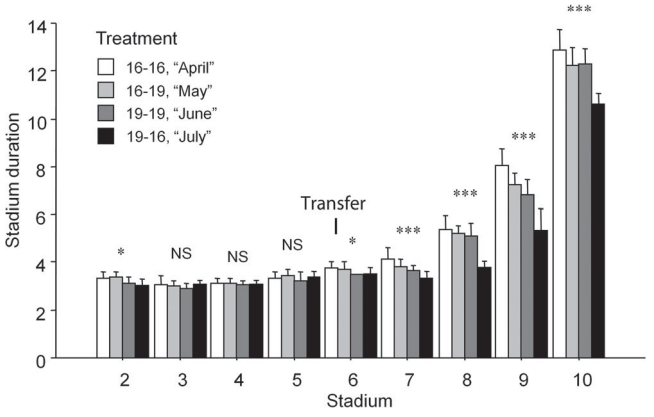

Figure 6. Repeated measures ANOVA for durations of each larval stadium in larvae completing development with 10 stadia in the four groups of experiment 2 (treatment: $F_{3,36} = 48.632$, $p < 0.001$, duration: $F_{8,288} = 2117.951$, $P < 0.001$; treatment × duration: $F_{24,288} = 11.148$, $p < 0.001$), followed by a posteriori ANOVAs between treatments for each single stadium. Error bars show SD. Symbols (tests for single stadia): NS not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Transfer mostly took place in early stadium 6 (Figure 5).

Analysis of larvae with 10 stadia in experiment 2

Looking at emergence dates in experiment 2, removal of the larvae with nine and 11 stadia did not dramatically reduce differences between treatments (Figure 5, black symbols). The remaining 10-stadia larvae can then be used for a more strict comparison of stadium durations between the four treatments (Figure 6). Repeated measures ANOVA revealed a significant treatment by duration interaction ($F_{24,288} = 11.148$, $p < 0.0001$), i.e. in the course of development the duration varied.

The first five moulting intervals (duration of stadia 2–6, mainly pre-transfer time), were all between three and four days and similar to each other and between treatments (although there were significant effects for stadia 2 and 6, Figure 6). In general, stadium duration increased steeply towards the end of development, F-0 taking (mean ± SD) 10.6 ± 0.4 (19-16; $n = 8$) to 12.9 ± 0.8 (16-16; $n = 9$) days (total span 10–14.5 days). Stadium durations after transfer mirrored the differences in emergence time/larval duration almost immediately, the photoperiodic decrease in the 19-16 group producing an outstandingly fast development.

During stadium 7, or F-3 in these 10-stadia larvae, differences between groups began to increase, and were significant ($p < 0.001$; Figure 6). The durations in the slow 16-16 group increased monotonously in the following stadia and were always the longest compared to the other groups. The 16-19 and 19-19 groups, then in the same LD 19.5:4.5 photoperiod, showed durations relatively similar to each other, increasing in an almost identical manner from stadium 7. The 19-16 group stands out from stadium 7 onwards, with even a slight decrease in the duration of stadium 7 compared to 6, and then increasing. Both absolute and relative differences between groups were highest in F-1. However, in experiment 1 the few larvae with 10 stadia did not indicate much of a difference between treatments (Figure 5a, b).

If F-0 was subdivided in the parts before and after the folding of the costal rib, a reasonable proxy of apolysis, shown in Figure 4a, it was seen that both parts contributed to the variation (ANOVA, $F_{3,39} = 10.21$, $p < 0.001$, and $F_{3,39} = 9.759$, $p < 0.001$, respectively; Figure 7). However, most of the observed differences in total F-0 duration between groups, and also most of the within-group variation, occurred during the period before folding (black in Figure 7). Only the most accelerated 19-16 group was standing out significantly from the other groups (Tukey's Q, $p < 0.01$). It is remarkable that there were significant, although small, differences between treatments also in the less variable post-apolysis part (grey in Figure 7). The 19-16 group was

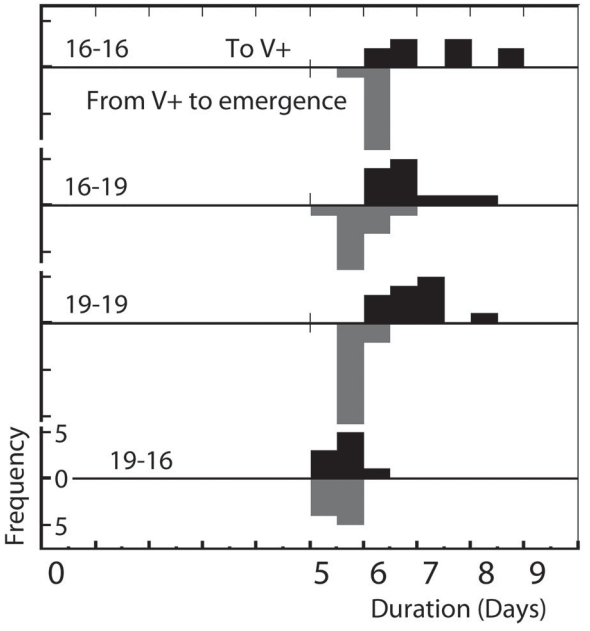

Figure 7. F-0 development before (black) and after (grey) folding of the costal rib (V +) in experiment 2. Also the four specimens with nine or 11 stadia are included.

significantly faster than the 16-16 and 16-19 groups only (Tukey's Q, $p < 0.01$), and the slow 16-16 also differed significantly from 19-19 (Tukey's Q, $p < 0.05$), but other combinations did not show significant differences. As seen in Figure 7, the costal folding started exactly half way through F-0 in the 19-16 group, but relatively later in the other ones.

Growth ratios

The growth ratio is here referred to as the increase in size at the ecdysis after a mentioned stadium, during which the subsequent stadium was generated. This differs from the notation in Corbet (2002).

For the 10-stadia larvae in experiment 2, repeated measures ANOVA on the 37 successfully emerging larvae revealed a significant treatment by ratio interaction ($F_{4,264} = 67.097$, $p < 0.0001$). However, except for the small stadia 2 and 3, where small errors in measurement can greatly affect the result (in fact significant differences; Supplementary Figure S3), growth ratios in 10-stadia larvae differed relatively little between treatment groups in experiment 2, despite differences in stadium duration. Only for F-0, during preparation for emergence, the fastest 19-16 group appeared to have the expected slightly lower growth ratio than the slower groups ($p < 0.001$). However, for F-1, where stadium durations differed the most, there was an unexpected reverse difference ($p < 0.05$). The overall tendency of the growth ratios in the larval moults was a dramatic and monotonous decrease from 1.56 ± 0.05 (from stadium 2) to 1.185 ± 0.011 (from stadium 9 or F-1), the last larva to larva moult. The ratio from F-0, at the imaginal moult (emergence), with accompanying reorganization, was higher, 1.257 ± 0.011.

In the sole larva with 11 stadia the growth ratios were generally lower, with the minimum also from stadium 9, which here was F-2. The range was 1.53 to 1.17, 1.19 from F-1, and 1.24 from F-0. Predictably, in the nine larvae with nine stadia (both experiments) growth ratios were generally

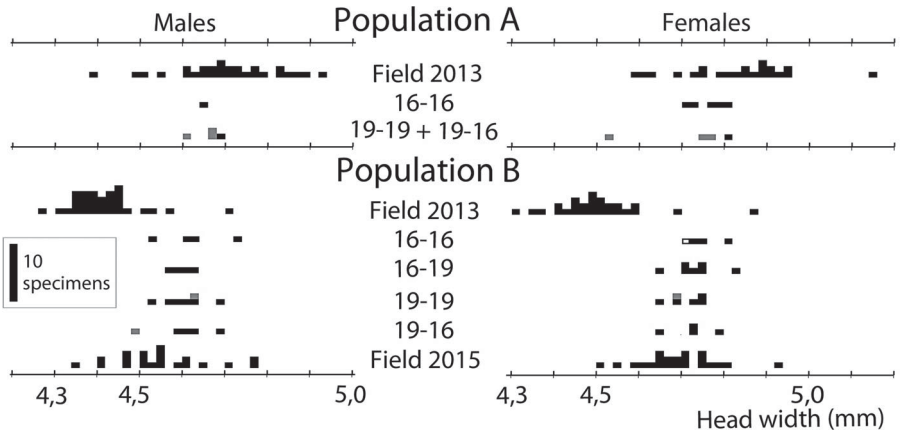

Figure 8. Sizes of adults from field samples and the different experimental groups. Number of stadia in experimental individuals are coded as in Figure 5.

somewhat higher, 1.596 ± 0.015 (a high value for dragonflies; Corbet, 2002) to 1.212 ± 0.014, and 1.259 ± 0.015 from F-0.

Size of adults

A summary of adult sizes in experiments and field samples is shown in Figure 8 and Supplementary Table S1. The field sampled population A adults were notably bigger than those from the nearby *Typha*-choked B site in 2013. In 2015, following the cleaning out of *Typha*, the population B adults were bigger, but still distinctly smaller than the population A 2013 adults.

In the experiments the population A adults (experiment 1) appeared just barely bigger than the experimental population B adults (experiment 2). Experimental adults from both populations fell within the range of the field collected ones from population A except for one small experiment 1 female (also population A). On the other hand, excepting this small female, all experimental adults were bigger than population B 2013 field adults except for some outliers, but in the range of the 2015 specimens.

Due to few individuals and sex differences, it is difficult to assess if there were any size differences between experimental treatments. At any rate, differences were small (Figure 8). The few nine-stadia larvae were not very different from other larvae, except that both the smallest female and smallest male belonged to this group. The 11-stadia specimen was relatively average.

Discussion

This study was performed with limited resources, imposing unwanted restrictions in the design of the experiments. Therefore the results must be regarded as tentative because they are based on few individuals. Also, the genetic homogeneity of the siblings used in the experiments makes the universality of the results in strong need of confirmation. However, the homogeneity should not weaken the significance of differences between treatments; rather the reverse. Due to the low numbers, and as a partial compensation, feeding, monitoring and measurements of all stadia could be meticulously carried out, providing important information not otherwise available.

Diapause termination and hatching time

The early and synchronous hatching in experiment 1 and 2, unaffected by treatment photoperiods and virtually finished within five days, indicated that termination of diapause was efficient, and eggs were in proper spring condition. This was in accordance with the data of Sawchyn & Church (1973) for the related *L. disjunctus* and *L. unguiculatus*. As eggs were subjected to LD 16:8 already during the late part of the cold storage (Figure 2a), the photoperiodically controlled phase 2 in diapause termination may also have been initiated. This late April photoperiod should be appropriate for hatching in the field, since hatching in central Europe, including Britain and Denmark, is universally reported to start during April (compilation in Jödicke, 1997, p. 151; see also Figure 1b–d). Also hatching in the field seems to take place within the span of a few days (Corbet, 1956a, b; Sternberg, 1999, p. 415 for *L. sponsa*, and Sawchyn & Church, 1973; Sawchyn & Gillott, 1974, for the Canadian relatives).

Thus, the fast and synchronous hatching of the eggs from both females at native spring photoperiods agrees with field observations. However, it is clearly in conflict with the consistently delayed spring hatching in some experiments with different, and shorter, winter treatments in darkness (Śniegula, Gołąb, & Johansson, 2015a, 2015b; Śniegula & Johansson, 2010). These questions are intended to be addressed in a separate communication in the light of recent findings and similarities with diapause maintenance and termination in many other insects (Tauber et al., 1986, p. 126–133).

Larval development

Larval development time and the timing of emergence (Figure 5) followed the predictions from the expected time constraints from the coarsely simulated seasons in experiment 2. This supports the hypothesis that both absolute photoperiods and changes in photoperiod are interacting in a most appropriate way to sense the phase of the photoperiodic regime during development. In experiment 2 the later stadia in the slow 16-16 group appeared to respond to LD 16:8 as if in spring, whereas those in the 19-16 (“July”) group responded to the same photoperiod as if it was signalling a time after the summer solstice. The latter group experienced an extreme time constraint, i.e. a shift to August photoperiods relatively early in life, which even could have been outside the range for relevant responses. This group stood out very distinctly as the fastest developing one in experiment 2. The differences between fast and slow groups were strongly related to moulting intervals in later stadia, but also to the total number of stadia.

Early in life the remaining development is less predictable and responses may occur only if time constraining cues are strong. For young larvae a summer solstice photoperiod is likely to be such a strong cue, and it may have been able to induce a lower number of stadia during early development, and so speed up development without changing moulting intervals. Development with nine stadia was only found in nine larvae starting at the long midsummer photoperiod, and these were all bigger in the sixth stadium than other larvae (Supplementary Figure S2). This is in accordance with the hypothesis where absolute photoperiods during early stadia should affect remaining development. However, the higher incidence of nine-stadia larvae in experiment 1 was correlated with a larger size of hatchlings, which could be genetic/maternal (single females were used), or an effect of different pre-treatment. It is also possible that an accidental initial difference in hatchling size between treatments in experiment 1 had affected the difference in stadium numbers (Supplementary Figure S2). Larger hatchlings were first shown to be correlated with fewer stadia and variation in development time in *Aeshna cyanea* (Müller) (Schaller, 1960). Also Johansson et al. (2001) found a correlation of a higher number of stadia in *L. sponsa* with slow development in a low food treatment. The numbers were stated as mostly eight and nine, but

as the prolarva was not included (Johansson, 2015, personal communication) this corresponds to nine and 10 in the present study.

Later in life larvae are closer to the deadline of emergence and are likely to have a stronger and more precise response to cues. The strongest effects of time stress were on moulting intervals during the last 3–4 stadia, at maximum in F-1, but even including post-apolysis development in F-0. In larvae developing with 10 stadia in experiment 2 (Figure 6), it was clearly seen that the almost drastic effect on development rate in the 19-16 group was limited to the period after the sudden decrease in photoperiod at transfer. Although much of these results and conclusions must be regarded as tentative due to the mentioned limitations, and the failure of the LD 16:8 switch detected five days after transfer, this is strong evidence that different photoperiods during early larval life can strongly affect the response to photoperiod later in life. In fact, the fast 19-16 larvae spent all the time after transfer, i.e. most of their life, together with the most slowly developing 16-16 group in the very same cabinet whatever the effect of the faulty switch in 16-16. At transfer time, all 10-stadia groups of larvae were still closely synchronous.

At the same time as constant long midsummer days (19-19) speed up development as compared to constant shorter spring days (16-16), decreasing late summer days (19-16) are still more accelerating than the long days. This means that the decrease appears to reinforce an overall accelerating effect of long days. This appears inverted, since the effect of such a decrease is normally reinforcing a short-day response, producing an effect of still shorter days, as shown in some other insects (e.g. Danks, 1987, p. 112; Nylin, 1989, 1992; Tauber et al., 1986, p. 123–124) and in Odonata for *L. dubia* (Norling, 1976, p. 251 and figure 3). As said, confirmation of these remarkable results is highly desirable. In addition, the outcome of the still smaller experiment 1 was partly different. However, when long days accelerate a development ending in mid-season, and short days accelerate late-season events (Nylin & Gotthard, 1998), it makes ecological sense to see these responses somehow combined when a potentially long and time constrained development can take place during both increasing and decreasing photoperiods.

De Block et al. (2008) showed that in the North American temporary pond species *L. congener* and *L. forcipatus*, the effect of a 15-day phase difference in photoperiodic regimes was noticeable at the entry into the final stadium, and it continued to increase until emergence, when it was *c.* three days. In the same study, the vernal pond specialist *L. dryas* had the whole effect of time restriction (also *c.* three days) confined to the final stadium, which was supposed to reflect its extreme commitment to early emergence, with an unregulated maximum rate in earlier stadia. In the present study, *Lestes sponsa*, a generalist pond species, had *c.* 25% of the total effect in F-0, and the larger total effect, *c.* eight days, was probably caused by a larger difference in time constraint.

Photoperiodic regimes in experiments

Previous studies using photoperiodic regimes rather than stationary photoperiods can shed further light on responses to changes versus absolute values of photoperiod, at the same time as the present kind of study may open up for an extended interpretation of this previous work.

Śniegula & Johansson (2010) compared Polish and north Swedish populations of *L. sponsa* in both Swedish and Polish photoperiodic regimes, although the used photoperiods were just sunrise to sunset, and may have been perceived as a bit short. Swedish eggs in the shorter Polish photoperiods hatched close to the summer solstice, almost a month later than in their native regime. This is very late in phase, but at photoperiods more like native early spring photoperiods for the Swedish population, but decreasing. Despite the late phase, development time of Swedish larvae was *c.* 16 days longer in Polish photoperiods than in Swedish, suggesting that

the longer Swedish days, despite a less time constraining phase, took precedence in determining development rate.

The probable accelerating effect of decreasing summer photoperiods may only operate in an interval appropriate for the population, and even decreasing Polish summer solstice photoperiods, in particular when just sunrise to sunset, may here produce an "early spring" response of low time restriction. This may be an example of an "outside the box" response. Another probable example is described in the introduction: "time stressed" and food deprived *Lestes sponsa* (Johansson, et al., 2001) developing slowly and emerging late as very large adults.

The solution of these problems must await more complete studies of the phenomenon. Remaining questions are, for example, the role of the rate of increase and decrease in photoperiod before and after the summer solstice, the timing of the photoperiodic changes during larval development, and not least the role of temperature in these responses. A low temperature should in principle be time constraining, but precludes any strong accelerating response.

Size variation in adults

It was expected to find a decrease in adult size correlating with high development rate, reflecting the commonly observed trade-off between size and time (Stoks et al., 2008). However, this could not be demonstrated with certainty (Figure 8). Possibly the rigorous twice-daily ad libitum feeding regime allowed for near complete compensation, at least for linear size (head width). If the larvae had been food limited the outcome might have been different.

Experimental adults have often been reported to be smaller than field collected adults from the same populations (e.g. Śniegula & Johansson, 2010; Śniegula et al., 2014). In the present study I partly encounter a reversed situation (Supplementary Table S1, Figure 8). Adults from experiment 1 were indeed slightly smaller than field specimens from the mother population (A), but the adults from experiment 2 were notably bigger than the 2013 field sample from the mother population (B). Either the offspring of the sole parent female was unusual, or, more likely, poor growth conditions in the particular environment of population B, the uniform *Typha*-choked roadside pond, was the cause. In 2015, when the pond had been opened up, field adults were more like those from the experiments, but still somewhat smaller. Some of the differences observed may, however, have been affected by different measurement methods, preservation artefacts and an about two-week earlier collection in 2015, but some differences are so great than this should not be the whole explanation. The smaller variation in size of the experimental adults (Figure 8, Supplementary Table S1) compared to field adults may reflect both uniform feeding, and a lack of genetic variation.

There seems to be a parallel situation to the size differences between the field samples in the present study and final stadium differences between the two relatively adjacent (*c*. 15 km) populations from the study of Pickup et al. (1984), shown in Figure 1b and c. The population in Figure 1c was, like the present population B, a dense *Typha* pond, and full-grown larvae were clearly smaller than in the Figure 1b population, a difference which will later carry over into the adults. In the British *Typha* pond, even the leading edge of development was distinctly slower than in the nearby population. Food limitation was suggested to be the cause of the small larvae, slow development and lack of synchrony at this locality (Pickup et al., 1984, p. 458). A possible late wetting of some eggs in *Typha* stalks, as discussed in the introduction, could also have contributed to time stressed development. Differences in adult size between different nearby field populations was also briefly mentioned by Śniegula & Johansson (2010), and were supposed to rely on environmental effects. Population differences, including the data from the present study, were also analysed in a different context by Śniegula, Gołab & Johansson (2016).

Food, development time and synchrony

In the present study development times were short in comparison with most other studies performed at a similar temperature (e.g. Johansson et al., 2001; Śniegula & Johansson, 2010; Śniegula et al., 2014). In addition, development was remarkably synchronous. Even the slowest specimen, an outlier with 11 stadia, emerging 53 days after hatching, was relatively fast compared to other studies. The shortest development time in any individual recorded by Śniegula and Johansson (2010) was 50 days, which is thus comparable with the longest durations in the present study. However, their temperature was 0.5°C, maybe even 1°C, lower, explaining part of the difference. Based on temperature summation (degree-days) above an assumed threshold of 9°C, reasonable if the temperature optimum is well above 21.5°C (cf. Suhling, Suhling & Richter, 2015), this would translate these 50 days into 48 and 46 days at 21.5°C, respectively, still a slow development (Table 1).

The fastest two specimens in experiment 1 completed development 35.5 days from hatching and 39 days after the start of experiment and are close to the record field observation of Schmidt (1993), when emergence started 37 days after water was introduced in a pond in Germany on 5 May. At a high food level, Fischer (1972) recorded 38 days from field caught hatchlings to emergence even at 20°C, which, roughly corrected for temperature, is close to the present results. However, her figure is very approximate due to high mortality and estimated age correlations made on later collected bigger larvae entering the experiments.

The remarkable development speed and synchrony in the otherwise typically asynchronous *L. sponsa* (compare Figure 1 and Supplementary Figure S1) is likely to be the result of homogenous controlled conditions during development (food, temperature, no competition), never occurring in the field, but also that larvae were the offspring of single females in each experiment. A possible greater synchrony in the groups with transfers (16-19 and 19-16) is at least consistent with shifts providing synchronizing signals of seasonal progress, absent during the constant conditions (Figure 5).

The differences from the results of many other studies concerning development time and adult size suggest that the common practice of *Artemia* feeding only once or less a day, often with a limited amount of metanaupliae dying after few hours in fresh water, introduces some amount of food limitation, in particular in bigger vials giving low food density. Poor emergence success is another likely effect of food restriction. When fed *Artemia* twice daily, larvae in the present study still had food in their guts from the previous meal, but nearly always vigorously attacked the new food items. It was also noted that at lower temperatures, *c*. 15°C, *Artemia* lived longer, up to 12 h, and at the same time larval metabolism and food requirements must be lower. Food limitation could thus increase with temperature, as also discussed by Suhling et al. (2015). However, there are likely to be other factors, as the shortest development time at 20°C was about 100 days in Johansson et al. (2001), without any apparent reason.

The relatively small vials and low water level in the present study could have simulated drying out of the pond, and so accelerated development (discussed in Stoks et al., 2008), and visual contact between larvae, which then may have perceived the presence of competitors, could also have affected development rate (e.g. Crowley, Gillett & Lawton, 1988).

Diapause and acceleration in larval development

The effect of different levels of time stress on development and growth rate in *Lestes* appears as a continuum of different development rates. Is this entirely different to an all or none, switch-like induction of diapause? However, a diapause response can be graded in intensity, and perhaps such a distinction is more apparent than real (Danks, 1987, p. 133; Nylin, Wickman, & Wiklund, 1989; Tauber et al., 1986). In Odonata a graded diapause response is common, and in summer

it is often a response to a varying degree of time surplus, delaying development just sufficiently (e.g. Norling, 1984c). In studies of Odonata with or without diapause, the period from the folding of the costal rib to emergence (most of the moult period in the moult–intermoult cycle of the last stadium) was essentially unaffected by photoperiod and diapause phenomena and dependent on temperature only, provided feeding was sufficient (Norling, 1976, 1984a–c). All variation in the F-0 stadium duration at a particular temperature occurred during the early part of the interecdysis period. In the present study, a small but distinct accelerating effect also on the time after costal folding was observed (Figure 7). This supports the notion that such acceleration of development is not only just the end of a continuum, with diapause standstill at the other end, but could at least partly be a different physiological phenomenon.

Towards an extended general model of seasonal regulation in larval Odonata

The two-step winter critical size model of seasonal regulation, mainly developed from studies at different latitudes in Sweden on species overwintering in the larval stage, presented by Norling (1984c), and more widely disseminated by Corbet (1999, p. 230), fail to incorporate univoltine species of the *Lestes*-type and bi- and multivoltine species. The present results open for an extended model, incorporating also these species. In this we have a successively increasing signal of time constraint, from spring to late summer. In the early season longer days are the cue, and after the solstice a decrease from the longest summer days can provide the continuation. This can produce a continuously variable timing of the initiation of a cohort split (critical time) from spring to late summer, depending on phenology and latitude. At this critical time an excessive time constraint causes larvae below a certain, often adaptable, critical size to enter a slow pre-hibernation development, aiming to reach a suitable overwintering stage and postpone emergence to the next season. The response pattern typical for the emerging, more or less time stressed group is reversed in these winter-bound larvae, which now instead have a time surplus. These divergent response patterns cause the cohort split.

The present findings could thus be the proximate explanation for late season cohort splitting in bi- and maybe multivoltine populations in temperate areas, best documented for *Enallagma aspersum* by Ingram & Jenner (1976b). A connection to a *Lestes* type life history can be seen in *Aeshna cyanea* in France, where growth was recorded under semi-natural aquarium conditions (natural photoperiod and 12–18°C over the year). Here a summer split produced a *Lestes*-like univoltine cohort and a semivoltine one with overwintering larvae (Schaller, 1960, figure 12, p. 780; see also Norling, 1984c, p.134). This is a typical two-step species in Sweden (Norling, 1984c, p 135, 139; 1971, p. 187).

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Supplemental data

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References

Artportalen. (2015). Swedish species observation system and virtual atlas. *Lestes sponsa*. Retrieved from http://www.artportalen.se

Beck, S. D. (1968). *Insect photoperiodism*. New York: Academic Press.

Billqvist, M., Smallshire, D., & Swash, A. (2012). *Svenska trollsländeguiden* [The Swedish dragonfly guide]. Malmö: Hirschfeld Media.
Corbet, P. S. (1956a). The life-histories of *Lestes sponsa* (Hansemann) and *Sympetrum striolatum* (Charp.) (Odonata). *Tijdschrift voor Entomologie*, *99*, 217–229.
Corbet, P. (1956b) The influence of temperature on diapause development in the dragonfly *Lestes sponsa* (Hansemann) (Odonata: Lestidae). Proceedings of the Royal Entomological Society of London, Series A, *General Entomology*, *31*, 45–48.
Corbet, P. S. (1956c). Environmental factors influencing the induction and termination of diapause in the emperor dragonfly, *Anax imperator* Leach (Odonata: Aeshnidae). *Journal of Experimental Biology*, *33*, 1–14.
Corbet, P. S. (1999). *Dragonflies: Behavior and ecology of Odonata*. Colchester: Harley Books.
Corbet, P. (2002). Stadia and growth ratios of Odonata: a review. *International Journal of Odonatology*, *5*, 45–73.
Corbet, P. S., & Harvey, I. F. (1989). Seasonal regulation in *Pyrrhosoma nymphula* (Sulzer) (Zygoptera: Coenagrionidae). 1. Seasonal development in nature. *Odonatologica*, *18*, 133–145.
Crowley, P. H., Gillett, S., & Lawton, J. H. (1988). Contests between larval damselflies: Empirical steps toward a better ESS model. *Animal Behaviour*, *36*(5), 1496–1510. doi:10.1016/S0003-3472(88)80220-3.
Danks, H. V. (1987). *Insect dormancy: An ecological perspective*. Ottawa: Biological Survey of Canada (Terrestrial Arthropods).
De Block, M., McPeek, M. A., & Stoks, R. (2008). Life-history evolution when *Lestes* damselflies invaded vernal ponds. *Evolution*, *62*, 485–493. doi:10.1111/j.1558-5646.2007.00283.x
De Block, M., & Stoks, R. (2003). Adaptive sex-specific life history plasticity to temperature and photoperiod in a damselfly. *Journal of Evolutionary Biology*, *16*, 986–995. doi:10.1046/j.1420-9101.2003.00581.x
Eller, J. G. (1963). *Seasonal regulation in* Pachydiplax longipennis *(Burmeister) (Odonata: Libellulidae)* (Unpublished doctoral dissertation). University North Carolina, Chapel Hill.
Fischer, Z. (1972). The energy budget of *Lestes sponsa* (Hans.) during its larval development. *Polish Archives of Hydrobiology*, *19*, 215–222.
Gotthard, K., Nylin, S., & Wiklund, C. (2000). Individual state controls temperature dependence in a butterfly (*Lasiommata maera*). *Proceedings of the Royal Society London*, *267*, 589–593. doi:10.1098/rspb.2000.1042.
Ingram, B. R. (1971). *The seasonal ecology of two species of damselflies (Odonata: Zygoptera) with special reference to the effects of photoperiod and temperature on nymphal development* (Unpublished doctoral dissertation). University North Carolina, Chapel Hill.
Ingram, B. R. (1975). Diapause termination in two species of damselflies. *Journal of Insect Physiology*, *21*, 1909–1016. doi:10.1016/0022-1910(75)90222-X
Ingram, B. R., & Jenner, C. E. (1976a). Influence of photoperiod and temperature on developmental time and number of moults in nymphs of two species of Odonata. *Canadian Journal of Zoology*, *54*, 2033–2045.
Ingram, B. R., & Jenner, C. E. (1976b). Life histories of *Enallagma hageni* (Walsh) and *E. aspersum* (Hagen) (Zygoptera: Coenagrionidae). *Odonatologica*, *5*, 331–345.
Johansson, F., & Rowe, L. (1999). Life history theory and behavioural responses to time constraints in a damselfly. *Ecology*, *80*, 1242–1252. doi:10.1890/0012-9658(2001)082[1857:LHPIAD]2.0.CO;2.
Johansson, F., Stoks, R., Rowe, L., & De Block, M. (2001). Life history plasticity in a damselfly: effects of combined time and biotic constraints. *Ecology*, *82*, 1857–1869. doi:10.1890/0012-9658(2001)082[1857:LHPIAD]2.0.CO;2
Jödicke, R. A. (1997). *Die Binsenjungfern und Winterlibellen Europas: Lestidae*. Magdeburg: Westarp Wissenschaften.
Lutz, P. E. (1968). Life-history studies on Lestes eurinus Say (Odonata). *Ecology*, *49*, 576–579.
Lutz, P. E. (1974). Effects of temperature and photoperiod on larval development in Tetragoneuria cynosura (Odonata: Libellulidae). *Ecology*, *55*, 370–377.
Lutz, P. E., & Jenner, C. E. (1964). Life-history and photoperiodic responses of nymphs of Tetragoneuria cynosura (Say). *The Biological Bulletin*, *127*, 304–316.
Mikolajewski, D. J., De Block, M., & Stoks, R. (2015). The interplay of adult and larval time constraints shapes species differences in larval life history. *Ecology*, *96*(4), 1128–1138. doi:10.1890/14-0262.1
Nilsson-Örtman, V., Stoks, R., De Block, M., & Johansson, F. (2012). Generalists and specialists along a latitudinal transect: patterns of thermal adaptation in six species of damselflies. *Ecology*, *93*, 1340–1352. doi:10.1890/11-1910.1
Norling, U. (1971). The life history and seasonal regulation of *Aeshna viridis* Eversm. in southern Sweden (Odonata). *Entomologica Scandinavica*, *2*, 170–190.
Norling, U. (1976). Seasonal regulation in *Leucorrhinia dubia* (Vander Linden) (Anisoptera: Libellulidae). *Odonatologica*, *5*, 245–263.
Norling, U. (1984a). The life cycle and larval photoperiodic responses of *Coenagrion hastulatum* (Charpentier) in two climatically different areas (Zygoptera: Coenagrionidae). *Odonatologica*, *13*, 429–449.
Norling, U. (1984b). Photoperiodic control of larval development in *Leucorrhinia dubia* (Vander Linden): a comparison between populations from northern and southern Sweden (Anisoptera: Libellulidae). *Odonatologica*, *13*, 529–550.
Norling, U. (1984c). Life history patterns in the northern expansion of dragonflies. *Advances in Odonatology*, *2*, 127–156.
Nylin, S. (1989). Effects of changing photoperiods in the life cycle regulation of the comma butterfly, *Polygonia c-album* (Nymphalidae). *Ecological Entomology*, *14*, 209–218. doi:10.1111/j.1365-2311.1989.tb00771.x
Nylin, S. (1992). Seasonal plasticity in life history traits: growth and development in *Polygonia c-album* (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society*, *47*, 301–323. doi:10.1111/j.1095-8312.1992.tb00672.x
Nylin, S., & Gotthard, K. (1998). Plasticity in life history traits. *Annual Review of Entomology*, *43*, 63–83. doi:10.1146/annurev.ento.43.1.63

Nylin, S., Wickman, P.-O., & Wiklund, C. (1989). Seasonal plasticity and development of the speckled wood butterfly. *Pararge aegeria* (Satyrinae). *Biological Journal of the Linnean Society*, *38*, 155–171. doi:10.1111/j.1095-8312.1989.tb01571.x

Pickup, J., Thompson, D. J., & Lawton, J. H. (1984). The life history of *Lestes sponsa* (Hansemann): Larval growth (Zygoptera: Lestidae). *Odonatologica*, *13*, 451–459.

Rota, E., & Carchini G. (1988). Considerations on an autumn record of *Lestes* larvae in Italy (Zygoptera: Lestidae). *Notulae Odonatologicae*, *3*, 9–13.

Sawchyn, W. W. (1972). *Environmental controls in the seasonal succession and synchronization of development in some pond species of damselflies (Odonata: Zygoptera)* (Unpublished doctoral dissertation). University of Saskatchewan, Saskatoon.

Sawchyn, W. W., & Church, N. S. (1973) The effect of temperature and photoperiod on diapause development in the eggs of four species of *Lestes* (Odonata: Zygoptera). *Canadian Journal of Zoology*, *51*, 1257–1265.

Sawchyn, W. W., & Gillott, C. (1974). The life histories of three species of *Lestes* (Odonata: Zygoptera) in Saskatchewan. *Canadian Entomologist*, *106*, 1283–1293.

Schaller, F. (1960). Étude du développement postembryonaire d'Aeschna cyanea Müll. *Annales des Sciences Naturelles Zoologique et Biologique Animales*, *2*, 751–868.

Schmidt, E. (1993). Die ökologische Nische von Sympetrum depressiusculum (Selys) im Münsterland (Naturschutzgebiet Heubachwiesen). *Libellula*, *12*, 175–198.

Śniegula, S., Drobniak, S. M., Gołąb, M. J., & Johansson, F. (2014). Photoperiod and variation in life history traits in core and peripheral populations in the damselfly *Lestes sponsa*. *Ecological Entomology*, *39*, 137–148. doi:1111/een.12076

Śniegula, S., Gołąb, M. J., & Johansson, F. (2015a). Time constraint effects on phenology and life history synchrony in a damselfly along a latitudinal gradient. *Oikos*, *125*, 414–423. doi:10.1111/oik.02265

Śniegula, S., Gołąb, M. J., & Johansson, F. (2015b). Data from: Time constraint effects on phenology and life history synchrony in a damselfly along a latitudinal gradient. – Dryad Digital Repository [Data set]. Retrieved from http://dx.doi.org/10.5061/dryad.7pr65

Śniegula, S., Gołąb, M. J., & Johansson, F. (2016). A large-scale latitudinal pattern of life-history traits in a strictly univoltine damselfly. *Ecological Entomology*, *41*, 259–472. doi:10.1111/een.12314.

Śniegula, S., & Johansson, F., (2010). Photoperiod affects compensating developmental rate across latitudes in the damselfly *Lestes sponsa*. *Ecological Entomology*, *35*, 149–57. doi:10.1111/j.1365-2311.2009.01164.x

Śniegula, S., Johansson, F., & Nilsson-Örtman, V. (2012a) Differentiation in developmental rate across geographic regions a photoperiod driven latitude compensating mechanism? *Oikos*, *121*, 1073–1082. doi:10.1111/j.1600-0706.2011.20015.x

Śniegula, S., Nilsson-Örtman, V., & Johansson, F. (2012b) Growth pattern responses to photoperiod across latitudes in a northern damselfly. *PLoS One*, *7*(9), e46024. doi:1371/journal.pone.0046024

Sternberg, K. (1999). *Lestes sponsa*. In K. Sternberg & R. Buchwald (Eds.), *Die Libellen Baden-Württembergs*, *Band 1* (pp. 409–418). Stuttgart: Ulmer.

Stoks, R., & De Block, M. (2011). Rapid growth reduces cold resistance: evidence from latitudinal variation in growth rate, cold resistance and stress proteins. *PLoS One*, *6*(2), e1695. doi:10.1371/journal.pone.0016935.

Stoks, R., Johansson F., & De Block M. (2008). Life history plasticity under time stress in damselfly larvae. In A. Cordoba-Aguilar (Ed.), *Dragonflies: Model Organisms for Ecological and Evolutionary Research* (pp. 39–51). Oxford: Oxford University Press.

Suhling, F., Suhling I., & Richter O. (2015). Temperature response of growth of larval dragonflies – an overview. *International Journal of Odonatology*, *18*, 15–30. doi:10.1080/13887890.2015.1009392.

Tauber, M. J., Tauber, C. A., & Masaki, S. (1986). *Seasonal adaptations of insects*. New York: Oxford University Press.

Uéda, T. (1978). Geographic variation in the life cycle of *Lestes sponsa*. *Tombo*, *21*, 27–34.

Uéda, T. (1989). Sexual maturation, body colour changes and increase of body weight in a summer diapause population of the damselfly *Lestes sponsa* (Hansemann) (Zygoptera: Lestidae). *Odonatologica*, *18*, 75–87.

Valtonen, P. (1982). [Observations of the life-cycle of Lestes sponsa (Odonata: Lestidae) in Finland] (in Finnish). *Notulae Entomologicae*, *62*, 151. [Odonatological Abstracts 4012; in *Odonatologica*, *12*, 194].

Warren, P. H. (1988). Larval overwintering in *Lestes sponsa* (Hans.) (Zygoptera: Lestidae). *Notulae Odonatologicae*, *3*, 15–17.